

Citation for published version:

Lopardo, L., Adams, D., Cummins, A. & Kasprzyk-Hordern, B 2018, 'Verifying community-wide exposure to endocrine disruptors in personal care products – In quest for metabolic biomarkers of exposure via in vitro studies and wastewater-based epidemiology', *Water Research*, vol. 143, pp. 117-126.
<https://doi.org/10.1016/j.watres.2018.06.028>

DOI:

[10.1016/j.watres.2018.06.028](https://doi.org/10.1016/j.watres.2018.06.028)

Publication date:

2018

Document Version

Peer reviewed version

[Link to publication](#)

Publisher Rights

CC BY-NC-ND

University of Bath

Alternative formats

If you require this document in an alternative format, please contact:
openaccess@bath.ac.uk

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Verifying community-wide exposure to endocrine disruptors in personal care products – in quest for metabolic biomarkers of exposure via *in vitro* studies and wastewater-based epidemiology

Luigi Lopardo, David Adams, Andrew Cummins and Barbara Kasprzyk-Hordern¹

¹Department of Chemistry, University of Bath, Bath BA2 7AY, UK

Abstract

This study aimed to identify human specific metabolites of selected known or suspected endocrine disruptors (EDCs), mainly UV filters, used in personal care and consumer products whose metabolism has hardly been explored and to select suitable candidate biomarkers for human exposure studies using wastewater based epidemiology (WBE). The analysis of metabolic biomarkers of target chemicals is crucial in order to distinguish between internal and external exposure, since many sources contribute to chemicals being discharged into wastewater. This was achieved through the employment of a new analytical framework for verification of biomarkers of exposure to chemicals combining human biomonitoring and water fingerprinting. Eight EDCs with unknown metabolic pathways (benzophenone-1 (BP-1); benzophenone-2 (BP-2); 4,4'-dihydroxybenzophenone (4,4'-DHBP); 4-benzylphenol (4-BenzPh); homosalate (HO); octocrylene (OC); 3-benzylidene camphor (3-BC), and two EDCs with known metabolism (bisphenol A (BPA) and benzophenone-3 (BP-3)) were tested. The biotransformation observed consisted mainly of phase I processes such as hydrolysis and hydroxylation together with phase II conjugation reactions such as sulphation and glucuronidation. Only two chemicals (BP-1, BP-3) were identified in urine and three chemicals (BPA, BP-1, BP-3) in wastewater. Five newly discovered metabolites (HO-Met1, OC-Met1, 4-BenzPh-Met4, 4-BenzPh-Met5 and 4-BenzPh-Met6) and one previously known metabolite (BPA-Met3) were detected in tested urine/wastewater samples from five WWTPs serving large communities ranging between 17 and 100 thousand inhabitants. The presence of metabolic biotransformation products of OC, 4-BenzPh, BPA and HO in wastewater provides evidence for internal exposure of studied populations to these chemicals.

Keywords: wastewater, epidemiology, exposure, UV filter, endocrine disruptor, personal care product, environment

1. Introduction

The last decades have seen a rapid increase in usage of personal care products and resultant public exposure to chemicals contained in those products (Calafat et al., 2015). Although the level of exposure to many of these chemicals might be low, the risk associated with it cannot be underestimated since simultaneous exposure to undefined mixtures might result in a synergistic effect making a comprehensive risk assessment process more complex compared to the assessment of a single chemical (Silins and Högberg, 2011). Long-term exposure leading to chronic effects should also not be underestimated. However very little is known about actual human exposure and therefore about the possibility to cause long term effects. UV filters are extensively used in a wide range of products including plastics, adhesives, rubber and personal care and consumer products including cosmetics, body lotions, hair sprays, skin creams, hair dyes or shampoos to preserve the integrity of the products (or skin, in the case of cosmetic sunscreens) from damage caused by the ultra-violet (UV) component of sunlight (particularly UVB, 290–320 nm). Some of these chemicals, their metabolites and/or their degradation products have been reported to be potentially bioaccumulative (Environment Agency, 2008), leading to endocrine disrupting effects (Krause et al., 2012; Zhao et al., 2013) and ecotoxicity in

¹Corresponding author: E-mail: B.Kasprzyk-Hordern@bath.ac.uk; Fax: +44(0) 1225 386231; Tel: +44 (0) 1225 385013

aquatic ecosystems (Díaz-Cruz and Barceló, 2009; Fent et al., 2008; Kaiser et al., 2012). In particular homosalate (HO), ethylhexylmethoxycinnamate (EHMC), octocrylene (OC) and benzophenone-3 (BP-3, regulated in the EU and commonly used in personal care products in the UK (Kerr, 2011)), have been demonstrated to exert endocrine disrupting effects *in vitro* and/or *in vivo*. According to Schlumpf et al. (Schlumpf et al., 2001) HO showed estrogenic effects *in vitro* (median effective concentration (EC₅₀) at 1.56 µM) and but not *in vivo* at similar concentrations, whereas EHMC has been proven to interfere with multiple endocrine pathways in rats (EC₅₀ 934 mg/kg/day). Seidlová-Wuttke et al. (Seidlová-Wuttke et al., 2006) observed a weak estrogenic effect of EHMC at high concentrations (275 mg/day for 6 weeks) while Klammer et al. (Klammer et al., 2007) observed an alteration of the hypothalamo-pituitary-thyroid function in the animals after 5 days of treatment. OC instead has been proven to exert antiestrogenic activity *in vitro* and *in vivo* in fish by Kunz et al., 2006 (Kunz and Fent, 2006a). Additionally some of these compounds are capable of damaging DNA via formation of radicals (Inbaraj et al., 2002).

Even though available information concerning the percutaneous absorption of UV filters in humans is still scarce, it is known that some of them can be absorbed through the skin (León-González et al., 2013), suggesting that exposure results mostly from topical application of personal care products (Ko et al., 2016). Moreover, Schlumpf et al. (Schlumpf et al., 2010) found a correlation between the presence of UV filters in human milk and the use of cosmetics, indicating that internal exposure resulted consistently from the usage of cosmetics. Ingestion of contaminated food and water (Loraine and Pettigrove, 2006; Wu et al., 2013) and inhalation of indoor dust represent other important indirect/environmental sources of exposure (Geens et al., 2009; Wang et al., 2013). The presence of those compounds has been also demonstrated in urine (Asimakopoulos et al., 2014; Schauer et al., 2006), wastewater and ultimately in the receiving environment as a consequence of their environmental persistence and widespread use (Gago-Ferrero et al., 2015; Gautam et al., 2014; Li et al., 2007; Poiger et al., 2004). Furthermore, given their physico-chemical properties, many of the compounds under investigation show great potential for bioaccumulation contributing to the formation of complex environmental mixtures raising public concern regarding their possible effects on human health and ecosystems (Balmer et al., 2005; Meyer et al., 2009). Dermal absorption of UV filters is considered to be one of the main routes of human exposure due to their extensive use in personal care and consumer products (León-González et al., 2013; Schauer et al., 2006; Schebb et al., 2011). Understanding toxicokinetic process, including metabolism, is therefore crucial in the determination of toxicological effects and bioaccumulation of these chemicals, as well as in the identification of biomarkers of exposure. Still there are only a few studies which reported the *in vivo* or *in vitro* biotransformation of UV filters. The metabolism of benzophenone-type UV filters has been investigated by Jeon et al. (Jeon et al., 2008) in rats observing mainly oxidative metabolites. These results were in accord with Watanabe et al. (Watanabe et al., 2015) who observed oxidative metabolites of BP-3 *in vitro*. Phase I and phase II metabolites of 3-(4'-methylbenzylidene)camphor (4-MBC) were found in urine, after oral administration in rats, as well as after dermal administration in both humans and rats (León-González et al., 2013; Schauer et al., 2006; Völkel et al., 2006). However, most other UV filters still remain hardly investigated.

This study aims to identify human specific metabolites of selected endocrine disruptors (EDCs), mainly UV filters, used in personal care and consumer products whose metabolism has hardly been explored and to select suitable candidate biomarkers of human exposure studies to these chemicals and for possible application in wastewater based epidemiology (WBE). The analysis of metabolic biomarkers of target chemicals is crucial in order to distinguish between internal and external exposure (e.g. direct disposal), since many sources contribute to chemicals being discharged into wastewater. This could be achieved through the employment of a new analytical framework (Fig.S4) for verification of biomarkers of exposure to chemicals combining human biomonitoring and water fingerprinting that was proposed by Lopardo et al. (Lopardo et al., 2017). Table S1 shows a list of eleven compounds that were prioritised in this study. The list includes nine chemicals (UV filters) used in personal care and consumer products, which are established or suspected EDCs and whose metabolism is yet unknown, and two compounds with known metabolism (bisphenol A (BPA) and benzophenone 3 (BP-3)).

2. Experimental section

2.1. Reagents and analytical standards

Water and methanol were of HPLC purity level and were purchased from Sigma-Aldrich. Pooled human liver microsomes (HLM), S9 fraction pooled from human liver, β -nicotinamide adenine dinucleotide 2'-phosphate reduced (β -NADPH $\geq 95\%$), alamethicin from *Trichoderma viride* ($\geq 98\%$), 3'-phosphoadenosine 5'-phosphosulphate lithium salt (PAPS, $\geq 60\%$), uridine 5'-diphosphoglucuronic acid trisodium salt (UDPGA, 98-100%), were purchased from Sigma-Aldrich (Gillingham, UK). The reference standards: 4-chloro-3-methylphenol (PCMC), 4-benzylphenol (4-BenzPh), bisphenol A (BPA), bisphenol A-D16, homosalate (HO), 3-benzylidene camphor (3BC), benzophenone-1 (BP-1), benzophenone-2 (BP-2), benzophenone-3 (BP-3), 4,4'-dihydroxybenzophenone (4,4'-DHBP), octocrylene (OC), potassium phosphate monobasic tetrasodium salt hydrate (KH_2PO_4), magnesium chloride hexahydrate (MgCl_2), were purchased from Sigma-Aldrich (Gillingham, UK). The following internal standards: 4-chloro-3-methylphenol-2,6-d2, triclosan-d3, benzophenone 3-d5 and bezafibrate-d6 were purchased from QMX Laboratories Ltd.

2.2. *In vitro* assays

Two step *in vitro* assay developed by Lopardo et al. (Lopardo et al., 2017), was undertaken in this study employing HLM and S9 fraction. 4-Cl-3-methylphenol was selected as a positive control to evaluate the performance of the assay. Two incubation mixtures were prepared in duplicate by mixing 10 μL of phosphate buffer (50 mM KH_2PO_4 , pH 7.4, 5 mM MgCl_2), 10 μL of analyte solution (50 μM), 10 μL of 100 μM UDPGA solution, and 10 μL of HLM spiked with 1 μL of an alamethicin solution (12.5 mg/mL). The reaction was initiated by the addition of 10 μL of a 10 mM NADPH solution followed by an incubation at 37 °C. The incubation was carried out for 3 hours under the same conditions for two of the four samples. At 3 hours, 10 μL of S9 fraction and 10 μL the 100 μM PAPS solution were added to the samples to be incubated for six hours and incubation was continued. The negative controls with either no analyte or no enzymes were prepared as well for each time point. The reaction was quenched with 100 μL of acetonitrile ice cold, followed by centrifugation at 10 000 rpm for 10 min (Centrifuge 5418, Eppendorf). The supernatant was removed and transferred to a new eppendorf tube and gently dried down by a stream of nitrogen at 40 °C using TurboVap evaporator (Caliper, UK). The resulting residue was reconstituted with 50 μL of a solution 80:20 H_2O /MeOH containing the internal standard (100 ng/mL), transferred into a polypropylene vial and analysed using Dionex Ultimate 3000 HPLC coupled with a Bruker Maxis HD Q-TOF according to the procedure described below. The range of metabolites produced and their relative ratios were investigated and compared (Table S2).

2.3. *In vivo* pooled urine essay

In vivo pooled urine essays were performed on seven pooled urine samples collected from a UK festival event. The samples were collected on three different days from five different urinals. Solid phase extraction (SPE) was performed using HLB Oasis® cartridges (Water, UK). For further details see Lopardo et al. (Lopardo et al., 2017). In addition HLB Oasis® cartridges, MCX and MAX Oasis® cartridges (Water, UK) were also used in this study. SPE extraction on the MCX and MAX cartridges was performed by loading 2 mL of pooled urine onto the cartridges, which were preconditioned with 2 mL MeOH (both cartridges) followed by 2 mL of H_2O with 2% formic acid on MCX and 2 mL of H_2O with 5% ammonium hydroxide on MAX. After loading, cartridges were dried for 30 min and elution was undertaken using 2 mL of MeOH (both cartridges), followed by 2 mL of MeOH with 7% ammonium hydroxide on MAX and 2 mL of MeOH with 2% formic acid on MCX. Extracts were dried using a TurboVap evaporator (Caliper, UK,) under a gentle nitrogen stream in a water bath at 40°C then reconstituted in 500 μL 80:20 H_2O :MeOH and analysed using Dionex Ultimate 3000 HPLC coupled with a Bruker Maxis HD Q-TOF according to the procedure described below.

2.4. Wastewater fingerprinting assay

24h composite (flow proportional) samples of raw wastewater (after physical screening) were collected from 5 different wastewater treatment plants and filtered using GF/F glass microfibre filter 0.75 μm (Fisher Scientific, UK). Solid phase extraction (SPE) was performed using HLB (Lopardo et al., 2017),

MCX and MAX Oasis® cartridges (Waters, UK). Extraction procedure included loading 100 mL of filtered wastewater onto Oasis cartridges which were preconditioned with 2 mL of MeOH for all cartridges followed by 2 mL of H₂O for HLB, 2 mL of H₂O with 2% formic acid for MCX and 2 mL of H₂O with 5% ammonium hydroxide for MAX. After loading the cartridges were dried for 30 minutes and analytes were eluted with 2 mL of MeOH for all cartridges followed by 2 more mL of MeOH for HLB cartridges, 2 mL of MeOH with 7% ammonium hydroxide for MCX and 2 mL of MeOH with 2% formic acid for MAX. Extracts were dried using a TurboVap evaporator (Caliper, UK,) under a gentle nitrogen stream in a water bath at 40°C then reconstituted in 250 µL 80:20 H₂O:MeOH and analysed using Dionex Ultimate 3000 HPLC coupled with a Bruker Maxis HD Q-TOF according to the procedure described below.

2.5. Liquid –chromatography coupled with tandem mass spectrometry

All samples were analysed with a Dionex Ultimate 3000 HPLC (Thermo Fisher UK Ltd.) coupled with a Bruker Maxis HD Q-TOF (Bruker) equipped with an electrospray ionization source. Nitrogen was used as nebulising gas at a flow rate of 11 L/min at a temperature of 220°C end at a pressure of 3 Bar. Capillary voltage was set at 4500 V and End Plate offset was set at 500 V. The method was applied both in ESI positive and negative and acquisition was performed in data independent broadband collision-induced dissociation acquisition mode (bbCID). In bbCID, precursor and product ion spectra were obtained by alternating low and high collision energy (respectively 7 and 20 eV). HyStar™ Bruker was used to coordinate the LC-MS system. Chromatographic separation of the metabolites formed was achieved by using an ACQUITY UPLC BEH C18 column (50 mm x 2.1 mm, 1.7 µm) and the following mobile phase composition: 1 mM ammonium fluoride in water (A) and methanol (B). The gradient elution both in ESI positive and negative mode was 5% B from 0 to 3 min and then increased for B as follows: 5–60% B from 3 to 4 min, followed by isocratic conditions at 60% B until 14 min, and 60–98% B from 14.1 to 17 min. The last step was a decrease of B 98%–5% from 17.1 to 20 min to re-equilibrate with the initial mobile phase composition before the next injection. The flow rate was kept constant at 0.4 ml/min and the column temperature was set at 40°C (see Lopardo et al., 2017). Method validation data are shown in Tables S3 and S4.

2.6. Biomarkers identification

Data extracted from the system after analysis of *in vitro* test samples were processed with MetID software from Advanced Chemistry Development, Inc. (ACD/Labs) for *in silico* prediction of suspected metabolite structures. The exact theoretical accurate mass of the structures was then associated to accurate masses found in the sample leading in case of a positive match to the identification of a chromatographic peak. The criteria for the elimination of false positives and the identification of actual metabolites amongst the numerous structures suggested were: (i) high mass accuracy (mass error below 5ppm for metabolites produced by *in vitro* studies and below 10 ppm for wastewater analysis) and (ii) lower K_{ow} compared to the non-metabolised compound. The chemical structure was then confirmed through the analysis of the fragmentation pattern (identification of fragments predicted by ACD/Labs MS fragmenter) and isotope pattern (when distinctive). The same workflow was then applied to confirm the presence in urine and wastewater of positively identified metabolites (Fig. S4). For details see (Lopardo et al., 2017).

3. Results and discussion

3.1. *In vitro* studies

A comprehensive listing of all observed metabolites, their masses, retention times and metabolite's respective identifying fragments generated at high energy (bbCID) mode is shown in Table S2. In most cases, at least one metabolite per UV filter was observed in *in vitro* studies. Both negative and positive ionisation modes were tested for the analysis, however all the potential metabolites resulted in better ion intensity in the negative ionization mode. The most common enzymatic biotransformation reactions observed for the UV filters investigated were hydroxylation, sulphate conjugation, glucuronide conjugation and combinations of hydroxylation and phase II biotransformation. The XIC of BP-1 Met2 (m/z 405.0827) produced two chromatographic peaks due to single hydroxylation occurring on two different positions, which lead to the identification of two different metabolites (Fig. S1, left). The XIC

of 4-BenzPh Met5 (m/z 279.0333) also produced two chromatographic peaks due to single hydroxylation occurring on two different positions, which lead to the identification of two different metabolites (Fig. S1, right). Enzymatic hydrolysis was observed only in the case of HO and OC that were broken down to smaller molecules that were reported not to be the products of photolysis (Karlsson et al., 2012; Rodil et al., 2009). All the metabolites produced have not been previously documented in literature.

3.2. *In vivo* pooled urine assay

The *in vivo* pooled urine assay led to identification of two metabolites that were also identified via *in vitro* studies: 4-BenzPh Met6 and OC Met1 (Table 1). The identification of the 4-BenzPh Met6 and OC Met1 in urine is reported in Figure 1. Figure 1a shows the XIC of 4-BenzPh twice hydroxylated and sulphated (m/z 295.0279; Fig. 1b) with elemental composition of the deprotonated molecule denoting $C_{13}H_{12}O_6S^-$ (1.0 ppm mass error). The analysis of the high-energy spectrum (bbCID mode; Fig. 1c) led to the identification of a fragment (m/z 215.0706) which corresponded with the loss of $[SO_3]$ from the precursor ion. Figure 1d shows the XIC of the hydrolytic metabolite of OC (m/z 248.0711; Fig. 1e) with elemental composition of the deprotonated molecule denoting $C_{15}H_{11}NO_2^-$ (2.4 ppm mass error). The analysis of the high-energy spectrum (bbCID mode; Fig. 1f) led to the identification of a fragment (m/z 204.0815) which corresponded with the loss of $[COO]$ from the precursor ion. Moreover, BP-3 and BP-1 were also identified in the analysed urine sample (Fig. S2). It is worth mentioning here that BP-1 can be also formed as a metabolite of BP-3. Interestingly, no BPA conjugated metabolites were observed in the analysed pooled urine samples.

3.3. Wastewater fingerprinting assay

Finally, the presence of the discovered metabolites was investigated in wastewater to selected biomarkers of public exposure to these chemicals. Analysis of untreated wastewater samples serving five large communities ranging between 17 and 100 thousand people was undertaken. The compounds detected in wastewater are summarised in Table 1. Among them are: BPA and BPA-Met2, BP-3, BP-1, HO-Met1, OC-Met1, 4-BenzPh-Met4 and 4-BenzPh-Met5.

Initial analysis of samples, performed using ACDLabs software, identified four potential metabolites: (1) the hydrolytic product of octocrylene (OC Met1), (2) the hydrolytic product of homosalate (HO Met1), (3) 4-benzylphenol sulphated (4-BenzPh-Met4) and (4) 4-benzylphenol sulphated and hydroxylated (4-BenzPh-Met5). The identification of the four metabolites is reported in Figures 2 and 3.

Figure 2a shows the XIC of HO Met1 (m/z 137.0247; Fig. 2b) with elemental composition of the deprotonated molecule denoting $C_7H_5O_3^-$ (2.2 ppm mass error). The analysis of the high energy spectrum (bbCID mode; Fig. 2c) led to the identification of a fragment (m/z 93.0350) which corresponded with the loss of $[C_9H_{18}]$ from the precursor ion. It is worth mentioning here that HO Met1, salicylic acid, has different application, e.g. as a pharmaceutical. Further work is needed to verify contributions from HO in the overall salicylic acid load in wastewater.

Figure 2d shows the XIC of OC Met1 (m/z 248.0735; Fig. 2e) with elemental composition of the deprotonated molecule denoting $C_{15}H_{11}NO_2^-$ (7.2 ppm mass error). The analysis of the high-energy spectrum (bbCID mode, Fig. 2f) led to the identification of a fragment (m/z 204.0834) which corresponded with the loss of $[COO]$ from the precursor ion.

Figure 3a shows the XIC of 4-BenzPh-Met4 (m/z 263.0408; Fig. 3b) with elemental composition of the deprotonated molecule denoting $C_{13}H_{11}O_4S^-$ (9.1 ppm mass error). The analysis of the high energy spectrum (bbCID mode; Fig. 3c) led to the identification of a fragment (m/z 183.0815) which corresponded with the loss of $[SO_3]$ from the precursor ion.

Figure 3d shows the XIC of 4-BenzPh-Met5 (m/z 279.0350; Fig. 2e) with elemental composition of the deprotonated molecule denoting $C_{13}H_{11}O_5S^-$ (6.1 ppm mass error). The analysis of the high-energy spectrum (bbCID mode, Fig. 3f) led to the identification of a fragment (m/z 199.0765) which corresponded with the loss of $[COO]$ from the precursor ion.

The relative intensities between the detected ions showed consistency compared to the *in vitro* experiments. BP-3, BP-1 (Fig. S3) and sulphated BPA (Fig. 4) were also identified in the analysed wastewater samples.

The presence of all the above metabolites detected in wastewater (including BPA sulphate) has not been previously documented in literature. As a result, there is no knowledge of the occurrence and effects of these chemicals in the context of environmental and public health. This study has proven that selected chemicals (4-benzylphenol, homosalate, octocrylene) used in personal care and consumer products undergo metabolism in humans leading to the formation of new chemicals that are found in communal wastewater and can be discharged into the receiving environment. Further work is required (a) to verify public exposure to EDCs utilising WBE and (b) to understand fate and biological effects of EDCs and their metabolites in exposed ecosystems.

To summarise, out of eleven chemicals were selected in this study, only two (BP-1, BP-3) were identified in urine and three (BPA, BP1, BP3) in wastewater. Five newly discovered metabolites (HO-Met1, OC-Met1, 4-BenzPh-Met4, 4-BenzPh-Met5 and 4-BenzPh-Met6) and one previously known metabolite (BPA-Met3) were detected in urine and/or wastewater. Such difference in detection patterns of selected EDCs and their metabolites was expected as:

- (1) They have different applications (Table S5), i.e. some (e.g. BP-3, HO, OC) are used mainly in personal care products and are applied directly to skin. Hence, due to direct exposure, they are more likely to be excreted with urine and discharged with wastewater as a result of e.g. showering.
- (2) They differ in frequency and volume of usage, i.e. only homosalate, octocrylene, and benzophenone-3 are in the top 10 of the most commonly used UV filters in the UK (Kerr, 2011). Their usage is season dependent.
- (3) Some could not be detected due to analytical constraints, e.g. HO, OC and 3-BC have higher hydrophobicity and could be more amenable to GC analysis.
- (4) Pooled urine and wastewater samples were collected at different time and location, therefore representing populations with different exposure signature.

The presence of BPA, OC and HO and 4-benzylphenol metabolic residues as well as the confirmation of benzophenones in urine and wastewater is of significant importance. For example, BPA and 4-BenzPh are mostly employed in the production of epoxy-phenolic resins and polycarbonate plastics used as thin protecting coatings on the interior surface of metal cans but it can be also found in many products like dental sealants, food packaging, beverage cans, personal care products, baby bottles, building materials and flame retardant materials. Consequently, there is a widespread potential for human exposure, which this study reports. Epidemiological studies along with animal and *in vitro* experiments reported BPA exposure as a potential cause of several adverse health effects, such as cancer, obesity and disorders in endocrine, renal and reproductive systems (Joint Fao Oms Expert Committee On Food Additives, 2010). Benzophenones, 4-BenzPh, OC and HO have been demonstrated in many studies to possess endocrine disrupting properties at rather high concentrations (Akahori et al., 2008; Fent et al., 2008; Kunz and Fent, 2006a; Schlumpf et al., 2001), but as highlighted by Kunz and Fent (Kunz and Fent, 2006b), for an adequate risk assessment when investigating endocrine disrupting properties, it becomes crucial to consider exposure to compound mixtures rather than single compounds given the possibility for a synergistic effects. Also, given their lipophilic nature, these compounds show great potential for bioaccumulation (Gago-Ferrero et al., 2015) and for ecotoxicological effects in aquatic ecosystems (Díaz-Cruz and Barceló, 2009; Fent et al., 2008; Kaiser et al., 2012).

4. Conclusions

This study presents a comprehensive examination of the *in vitro* metabolism of eleven EDCs used in personal care and consumer products (Table S5) in order to identify suitable biomarkers of exposure and their analysis in biological matrices such as urine and wastewater, and ultimately to verify the extent

of public internal and external exposure to these chemicals. External exposure indicates the whole EDC dose to which an organism was exposed. Internal exposure indicates only the fraction of the initial chemical dose that was absorbed by and distributed throughout the body. The biotransformation observed were mainly phase I processes such as hydrolysis and hydroxylation together with phase II conjugation reactions such as sulphation and glucuronidation. Eleven chemicals were selected in this study. Only two (BP-1, BP-3) were identified in urine and three (BPA, BP1, BP3) in wastewater. Five newly discovered metabolites (HO-Met1, OC-Met1, 4-BenzPh-Met4, 4-BenzPh-Met5 and 4-BenzPh-Met6) and one previously known metabolite (BPA-Met3) were detected in urine and/or wastewater.

This new approach towards biomarkers selection shows a significant potential, especially in its future application in verification of public exposure to chemicals using WBE. Furthermore, an opportunity for further studies focussing on understanding of fate and effects of EDCs and their metabolites in the aquatic environment needs to be emphasised too. It is apparent that further work is needed to increase the pool of available biomarkers, increase selectivity and sensitivity of analytical methods, and analyse larger sets of samples allowing for the verification of spatial and temporal changes in exposure patterns to chemicals. When combined with further studies in this area, researchers will have a basis for fine tuning *in vitro* assays to better produce results useful for comprehensive metabolic profiling of both known and novel chemicals.

Supporting information

The supporting information section contains:

List of tables

Table S1 List of compounds selected and their physical-chemical properties (<https://scifinder.cas.org>)

Table S2 List of metabolites produced via *in vitro* studies, suggested structures and relative abundances. Accurate masses, retention times and confirmation fragments structures produced in MS/MS mode are also provided.

Table S3 UHPLC-QTOF instrument performance parameters

Table S4 SPE-UHPLC-QTOF method performance parameters

Table S5 Purpose, commercial use and potential exposure sources of the selected compounds

List of figures

Figure S1 XIC at m/z 405.0827 (0.005-Da mass-window width) (a) and mass spectra of the peak eluted at 6.4 minutes (b) and of the peak eluted at 6.6 minutes (c). XIC at m/z 279.0333 (0.005-Da mass-window width) (d) and mass spectra of the peak eluted at 6.5 minutes (b) and of the peak eluted at 6.9 minutes (c).

Figure S2 Detection and identification of BP-1 (m/z 213.0557) and BP-3 (229.0859) by UHPLC-QTOF-MS following analysis of standard solutions and *in vivo* pooled urine assay. (a) XIC at and m/z 213.0557 (0.005-Da mass-window width) of a BP-1 standard solution; (b) XIC at and m/z 213.0557 (0.005-Da mass-window width) of a pooled urine sample and mass spectra (c). (d) XIC at m/z 229.0859 (0.005-Da mass-window width) of a BP-3 standard solution; (e) XIC at and m/z 229.0859 (0.005-Da mass-window width) of a pooled urine sample and mass spectra (f).

Figure S3 Detection and identification of BP-1 (m/z 213.0557) and BP-3 (229.0859) by UHPLC-QTOF-MS following analysis of standard solutions and wastewater fingerprinting assay. (a) XIC at and m/z 213.0557 (0.005-Da mass-window width) of a BP-1 standard solution; (b) XIC at and m/z 213.0557 (0.005-Da mass-window width) of a wastewater sample and mass spectra (c). (d) XIC at m/z 229.0859 (0.005-Da mass-window width) of a BP-3 standard solution; (e) XIC at and m/z 229.0859 (0.005-Da mass-window width) of a wastewater sample and mass spectra (f).

Figure S4 A systematic workflow for identification and selection of human biomarkers of exposure to environmental contaminants via combined *in vitro*, pooled urine and pooled wastewater profiling assay

List of reports

Report 1 (p.S8) XICs and mass spectra of BPA metabolites identified following *in vitro* experiments
Report 2 (p.S25) XICs and mass spectra of BP1 metabolites identified following *in vitro* experiments
Report 3 (p.S55) XICs and mass spectra of BP2 metabolites identified following *in vitro* experiments
Report 4 (p.S61) XICs and mass spectra of 4,4-DHBP metabolites identified following *in vitro* experiments
Report 5 (p.S82) XICs and mass spectra of 4-BenzPh metabolites identified following *in vitro* experiments
Report 6 (p.S115) XICs and mass spectra of HO metabolites identified following *in vitro* experiments
Report 7 (p.S120) XICs and mass spectra of OC metabolites identified following *in vitro* experiments
Report 8 (p.S125) XICs and mass spectra of 3BC metabolites identified following *in vitro* experiments
Report 9 (p.S129) XICs and mass spectra of compounds identified in urine
Report 10 (p.S143) XICs and mass spectra of compounds identified in wastewater

Acknowledgments

The support of the Leverhulme Trust (Project No RPG-2013-297) is greatly appreciated. We would also like to acknowledge Trevor Shine at TICTAC Communications (St George's University of London, United Kingdom) for provision of pooled urine samples. All data supporting this study are provided as supporting information accompanying this paper.

References

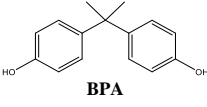
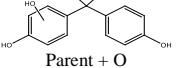
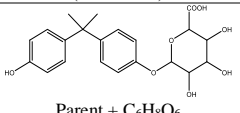
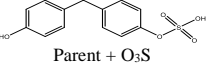
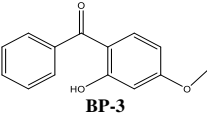
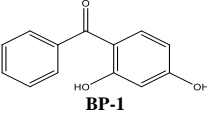
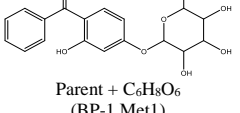
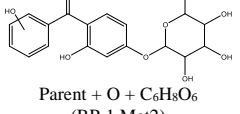
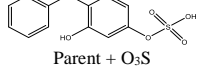
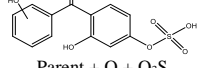
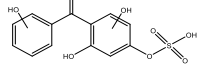

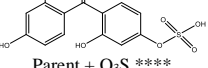
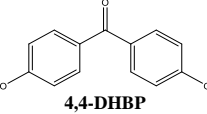
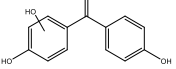
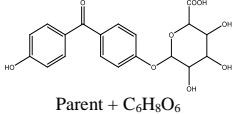
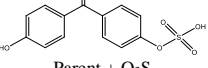
- Akahori, Y., Nakai, M., Yamasaki, K., Takatsuki, M., Shimohigashi, Y., Ohtaki, M., 2008. Relationship between the results of *in vitro* receptor binding assay to human estrogen receptor α and *in vivo* uterotrophic assay: Comparative study with 65 selected chemicals. *Toxicol. Vitro*. 22, 225–231. <https://doi.org/10.1016/j.tiv.2007.08.004>
- Asimakopoulou, A.G., Thomaidis, N.S., Kannan, K., 2014. Widespread occurrence of bisphenol A diglycidyl ethers, p-hydroxybenzoic acid esters (parabens), benzophenone type-UV filters, triclosan, and triclocarban in human urine from Athens, Greece. *Sci. Total Environ.* 470, 1243–1249. <https://doi.org/10.1016/j.scitotenv.2013.10.089>
- Balmer, M.E., Buser, H., Mu, M.D., Poiger, T., Faw, A., Federal, S., 2005. Occurrence of Some Organic UV Filters in Wastewater, in Surface Waters, and in Fish from Swiss Lakes. *Environ. Sci. Technol* 39, 953–962.
- Calafat, A.M., Valentin-Blasini, L., Ye, X., 2015. Trends in Exposure to Chemicals in Personal Care and Consumer Products. *Curr. Environ. Heal. reports* 2, 348–355. <https://doi.org/10.1007/s40572-015-0065-9>
- Díaz-Cruz, M.S., Barceló, D., 2009. Chemical analysis and ecotoxicological effects of organic UV-absorbing compounds in aquatic ecosystems. *TrAC - Trends Anal. Chem.* 28, 708–717. <https://doi.org/10.1016/j.trac.2009.03.010>
- Environment Agency, 2008. UV-filters in cosmetics – prioritisation for environmental assessment, Science.
- Fent, K., Kunz, P.Y., Gomez, E., 2008. UV Filters in the Aquatic Environment Induce Hormonal Effects and Affect Fertility and Reproduction in Fish. *Chim. Int. J. Chem.* 62, 368–375. <https://doi.org/10.2533/chimia.2008.368>
- Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2015. UV filters bioaccumulation in fish from Iberian

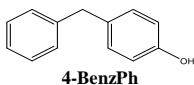
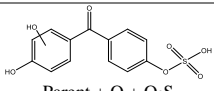
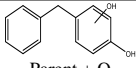
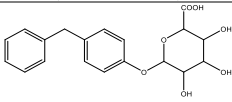
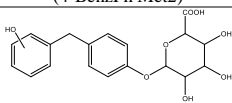
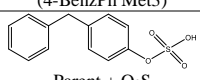
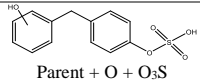
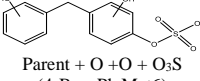
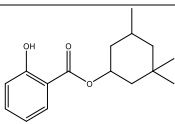
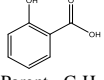
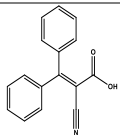
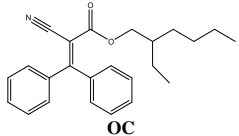
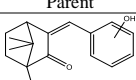
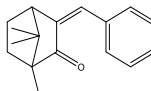
- river basins. *Sci. Total Environ.* 518-519, 518–525.
<https://doi.org/10.1016/j.scitotenv.2015.03.026>
- Gautam, P., Carsella, J.S., Kinney, C.A., 2014. Presence and transport of the antimicrobials triclocarban and triclosan in a wastewater-dominated stream and freshwater environment. *Water Res.* 48, 247–256. <https://doi.org/10.1016/j.watres.2013.09.032>
- Geens, T., Roosens, L., Neels, H., Covaci, A., 2009. Assessment of human exposure to Bisphenol-A, Triclosan and Tetrabromobisphenol-A through indoor dust intake in Belgium. *Chemosphere* 76, 755–760. <https://doi.org/10.1016/j.chemosphere.2009.05.024>
- Gonzalez, H., Jacobson, C., Wennberg, A., Larkö, O., Farbroth, A., 2008. Solid-Phase Extraction and Reverse-Phase HPLC : Application to Study the Urinary Excretion Pattern of Benzophenone-3 and its Metabolite 2 , 4-Dihydroxybenzophenone in Human Urine. *Anal. Chem. Insights* 3, 1–7.
- Inbaraj, J.J., Bilski, P., Chignell, C.F., 2002. Photophysical and photochemical studies of 2-phenylbenzimidazole and UVB sunscreen 2-phenylbenzimidazole-5-sulfonic acid. *Photochem. Photobiol.* 75, 107–116. [https://doi.org/10.1562/0031-8655\(2002\)075<0107:PAPSOP>2.0.CO;2](https://doi.org/10.1562/0031-8655(2002)075<0107:PAPSOP>2.0.CO;2)
- Jeon, H.-K., Sarma, S.N., Kim, Y.-J., Ryu, J.-C., 2008. Toxicokinetics and metabolisms of benzophenone-type UV filters in rats. *Toxicology* 248, 89–95.
<https://doi.org/10.1016/j.tox.2008.02.009>
- Joint Fao Oms Expert Committee On Food Additives, 2010. Toxicological and Health Aspects of Bisphenol A Joint FAO / WHO Expert Meeting. Fao Who 60.
- Kaiser, D., Sieratowicz, A., Zielke, H., Oetken, M., Hollert, H., Oehlmann, J., 2012. Ecotoxicological effect characterisation of widely used organic UV filters. *Environ. Pollut.* 163, 84–90.
<https://doi.org/10.1016/j.envpol.2011.12.014>
- Karlsson, I., Persson, E., Mårtensson, J., Börje, A., 2012. Investigation of the sunscreen octocrylene's interaction with amino acid analogs in the presence of UV radiation. *Photochem. Photobiol.* 88, 904–912. <https://doi.org/10.1111/j.1751-1097.2012.01142.x>
- Kerr, A.C., 2011. A survey of the availability of sunscreen filters in the UK. *Clin. Exp. Dermatol.* 36, 541–543. <https://doi.org/10.1111/j.1365-2230.2010.04007.x>
- Klammer, H., Schlecht, C., Wuttke, W., Schmutzler, C., Gotthardt, I., Köhrle, J., Jarry, H., 2007. Effects of a 5-day treatment with the UV-filter octyl-methoxycinnamate (OMC) on the function of the hypothalamo-pituitary-thyroid function in rats. *Toxicology* 238, 192–199.
<https://doi.org/10.1016/j.tox.2007.06.088>
- Ko, A., Kang, H.S., Park, J.H., Kwon, J.E., Moon, G.I., Hwang, M.S., Hwang, I.G., 2016. The Association between Urinary Benzophenone Concentrations and Personal Care Product Use in Korean Adults. *Arch. Environ. Contam. Toxicol.* 70, 640–646. <https://doi.org/10.1007/s00244-015-0240-x>
- Krause, M., Klit, A., Blomberg Jensen, M., Sjøborg, T., Frederiksen, H., Schlumpf, M., Lichtensteiger, W., Skakkebaek, N.E., Drzewiecki, K.T., 2012. Sunscreens: Are they beneficial for health? An overview of endocrine disrupting properties of UV-filters. *Int. J. Androl.* 35, 424–436.
<https://doi.org/10.1111/j.1365-2605.2012.01280.x>
- Kunz, P.Y., Fent, K., 2006a. Multiple hormonal activities of UV filters and comparison of in vivo and in vitro estrogenic activity of ethyl-4-aminobenzoate in fish. *Aquat. Toxicol.* 79, 305–324.
<https://doi.org/10.1016/j.aquatox.2006.06.016>
- Kunz, P.Y., Fent, K., 2006b. Estrogenic activity of UV filter mixtures. *Toxicol. Appl. Pharmacol.* 217, 86–99. <https://doi.org/10.1016/j.taap.2006.07.014>

- León, Z., Chisvert, A., Tarazona, I., Salvador, A., 2010. Solid-phase extraction liquid chromatography-tandem mass spectrometry analytical method for the determination of 2-hydroxy-4-methoxybenzophenone and its metabolites in both human urine and semen. *Anal. Bioanal. Chem.* 398, 831–43. <https://doi.org/10.1007/s00216-010-3947-6>
- León-González, Z., Chisvert, A., Fernández, I., Salvador, A., 2013. A solid-phase extraction liquid chromatography-tandem mass spectrometry method for the percutaneous absorption assessment of 3-(4'-methylbenzylidene)camphor via human urine analysis. *Anal. Methods* 5, 367. <https://doi.org/10.1039/c2ay25490e>
- Li, W., Ma, Y., Guo, C., Hu, W., Liu, K., Wang, Y., Zhu, T., 2007. Occurrence and behavior of four of the most used sunscreen UV filters in a wastewater reclamation plant. *Water Res.* 41, 3506–12. <https://doi.org/10.1016/j.watres.2007.05.039>
- Lopardo, L., Cummins, A., Rydevik, A., Kasprzyk-Hordern, B., 2017. New Analytical Framework for Verification of Biomarkers of Exposure to Chemicals Combining Human Biomonitoring and Water Fingerprinting. *Anal. Chem.* 89, 7232–7239. <https://doi.org/10.1021/acs.analchem.7b01527>
- Loraine, G. a., Pettigrove, M.E., 2006. Seasonal variations in concentrations of pharmaceuticals and personal care products in drinking water and reclaimed wastewater in Southern California. *Environ. Sci. Technol* 40, 687–695. <https://doi.org/10.1021/es051380x>
- Meyer, V., Haase, D., Scheuer, S., 2009. Flood risk assessment in European river basins--concept, methods, and challenges exemplified at the Mulde River. *Integr. Environ. Assess. Manag.* 5, 17–26. <https://doi.org/10.1897/IEAM>
- Poiger, T., Buser, H.-R., Balmer, M.E., Bergqvist, P.-A., Müller, M.D., 2004. Occurrence of UV filter compounds from sunscreens in surface waters: regional mass balance in two Swiss lakes. *Chemosphere* 55, 951–63. <https://doi.org/10.1016/j.chemosphere.2004.01.012>
- Rodil, R., Moeder, M., Altenburger, R., Schmitt-Jansen, M., 2009. Photostability and phytotoxicity of selected sunscreen agents and their degradation mixtures in water. *Anal. Bioanal. Chem.* 395, 1513–1524. <https://doi.org/10.1007/s00216-009-3113-1>
- Schauer, U.M.D., Völkel, W., Heusener, A., Colnot, T., Broschard, T.H., von Landenberg, F., Dekant, W., 2006. Kinetics of 3-(4-methylbenzylidene)camphor in rats and humans after dermal application. *Toxicol. Appl. Pharmacol.* 216, 339–46. <https://doi.org/10.1016/j.taap.2006.05.011>
- Schebb, N.H., Inceoglu, B., Ahn, K.C., Morisseau, C., Gee, S.J., Hammock, B.D., 2011. Investigation of Human Exposure to Triclocarban after Showering and Preliminary Evaluation of Its Biological Effects. *Environ. Sci. Technol* 45, 3109–3115.
- Schlumpf, M., Cotton, B., Conscience, M., Haller, V., Steinmann, B., Lichtensteiger, W., 2001. In vitro and in vivo estrogenicity of UV screens. *Environ. Health Perspect.* 109, 239–244. <https://doi.org/10.1289/ehp.109-a359>
- Schlumpf, M., Kypke, K., Wittassek, M., Angerer, J., Mascher, H., Mascher, D., Vökt, C., Birchler, M., Lichtensteiger, W., 2010. Exposure patterns of UV filters, fragrances, parabens, phthalates, organochlor pesticides, PBDEs, and PCBs in human milk: Correlation of UV filters with use of cosmetics. *Chemosphere* 81, 1171–1183. <https://doi.org/10.1016/j.chemosphere.2010.09.079>
- Seidlová-Wuttke, D., Jarry, H., Christoffel, J., Rimoldi, G., Wuttke, W., 2006. Comparison of effects of estradiol (E2) with those of octylmethoxycinnamate (OMC) and 4-methylbenzylidene camphor (4MBC) - 2 filters of UV light - On several uterine, vaginal and bone parameters. *Toxicol. Appl. Pharmacol.* 210, 246–254. <https://doi.org/10.1016/j.taap.2005.05.006>
- Silins, I., Högberg, J., 2011. Combined toxic exposures and human health: Biomarkers of exposure

- and effect. *Int. J. Environ. Res. Public Health* 8, 629–647.
<https://doi.org/10.3390/ijerph8030629>
- Völkel, W., Colnot, T., Schauer, U.M.D., Broschard, T.H., Dekant, W., 2006. Toxicokinetics and biotransformation of 3-(4-methylbenzylidene)camphor in rats after oral administration. *Toxicol. Appl. Pharmacol.* 216, 331–8. <https://doi.org/10.1016/j.taap.2006.05.012>
- Wang, L., Asimakopoulos, A.G., Moon, H.B., Nakata, H., Kannan, K., 2013. Benzotriazole, benzothiazole, and benzophenone compounds in indoor dust from the United States and East Asian countries. *Environ. Sci. Technol.* 47, 4752–4759. <https://doi.org/10.1021/es305000d>
- Watanabe, Y., Kojima, H., Takeuchi, S., Uramaru, N., Sanoh, S., Sugihara, K., Kitamura, S., Ohta, S., 2015. Metabolism of UV-filter benzophenone-3 by rat and human liver microsomes and its effect on endocrine-disrupting activity. *Toxicol. Appl. Pharmacol.* 282, 119–128.
<https://doi.org/10.1016/j.taap.2014.12.002>
- Wu, X., Ernst, F., Conkle, J.L., Gan, J., 2013. Comparative uptake and translocation of pharmaceutical and personal care products (PPCPs) by common vegetables. *Environ. Int.* 60, 15–22.
<https://doi.org/10.1016/j.envint.2013.07.015>
- Ye, X.Y., Zsuzsanna, K., Needham, L.L., Calafat, A.M., 2005. Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* 383, 638–644. <https://doi.org/10.1007/s00216-005-0019-4>
- Zhao, H., Wei, D., Li, M., Du, Y., 2013. Substituent contribution to the genotoxicity of benzophenone-type UV filters. *Ecotoxicol. Environ. Saf.* 95, 241–246.
<https://doi.org/10.1016/j.ecoenv.2013.05.036>

Table 1 Presence of the metabolites identified via *in vitro* studies in the analysed matrices

Compound	Metabolites identified via <i>in vitro</i> studies	Matrices					
		Urine	June WW	July WW	August WW	September WW	October WW
 BPA	Parent	✓*	< LOD	✓	✓	✓	✓
	 Parent + O (BPA Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + C ₆ H ₈ O ₆ (BPA Met2)	✓	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O ₃ S (BPA Met3)	✓	< LOD	✓	< LOD	✓	✓
 BP-3	Parent(Ye et al., 2005)	✓	✓	✓	✓	✓	✓
	BP-1(Gonzalez et al., 2008; León et al., 2010)	✓	✓	✓	✓	✓	✓
 BP-1	Parent	✓*	✓	✓	✓	✓	✓
	 Parent + C ₆ H ₈ O ₆ (BP-1 Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O + C ₆ H ₈ O ₆ (BP-1 Met2)	✓	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O ₃ S (BP-1 Met3)	✓	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O + O ₃ S (BP-1 Met4)	✓	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O + O + O ₃ S **** (BP-1 Met5)	✓	< LOD	< LOD	< LOD	< LOD	< LOD
 BP-2	Parent	✓*	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O ₃ S **** (BP-2 Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD
 4,4-DHBP	Parent	✓*	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O (4,4-DHBP Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + C ₆ H ₈ O ₆ (4,4-DHBP Met2)	✓	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O ₃ S (4,4-DHBP Met3)	✓	< LOD	< LOD	< LOD	< LOD	< LOD

 4-BenzPh	 Parent + O + O ₃ S (4,4-DHBP Me4)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	Parent	✓*	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O (4-BenzPh Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + C ₆ H ₈ O ₆ (4-BenzPh Met2)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O + C ₆ H ₈ O ₆ (4-BenzPh Met3)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O ₃ S (4-BenzPh Met4)	✓	< LOD	< LOD	< LOD	✓	< LOD	< LOD
	 Parent + O + O ₃ S (4-BenzPh Met5)	✓	< LOD	✓	✓	✓	< LOD	✓
	 Parent + O + O ₃ S (4-BenzPh Met6)	✓	✓	< LOD	< LOD	< LOD	< LOD	< LOD
	Parent	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
 HO	 Parent - C ₉ H ₁₆ (HO Met1)	✓	< LOD	✓	✓	✓	✓	✓
	Parent	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	 Parent - C ₈ H ₁₆ (OC Met1)	✓	✓	✓	✓	✓	✓	✓
 OC	Parent	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	 Parent + O (3-BC Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 3-BC							

*present as non-metabolized residue

** n.d. (non detectable)

*** WW (wastewater)

****same accurate mass and retention time

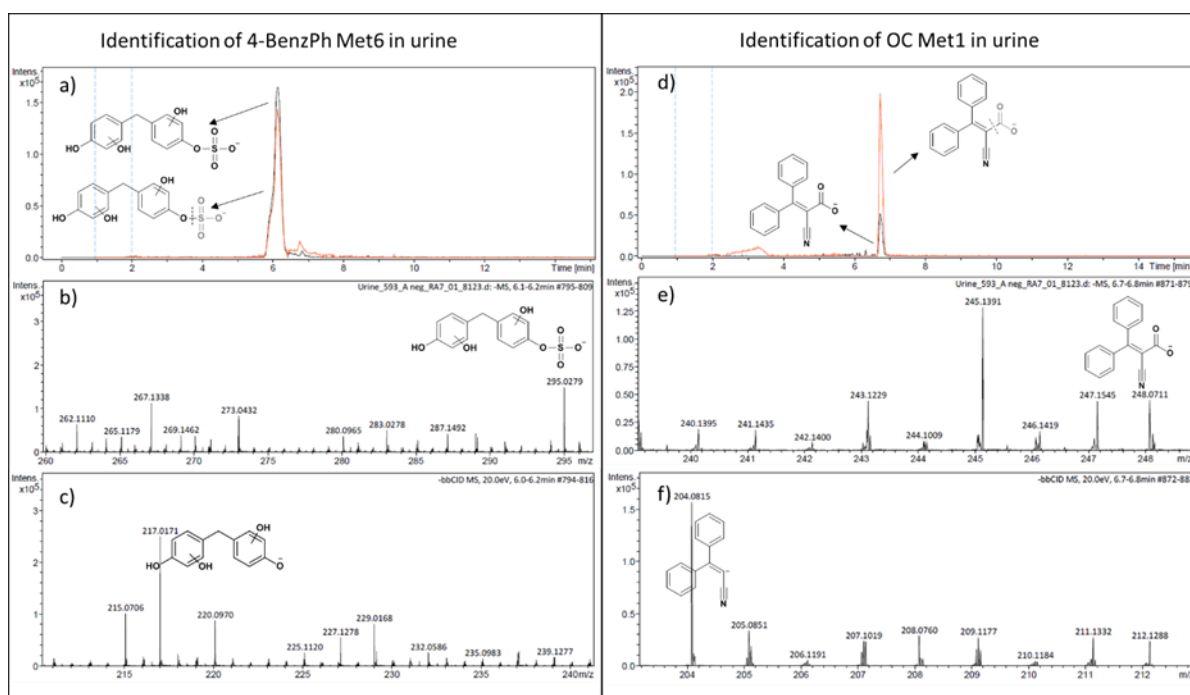


Figure 1 Detection and identification of 4-BenzPh Met6 (a;b;c) and OC Met1 (d;e;f) by UHPLC-QTOF-MS following *in vivo* pooled urine assay. (a) XICs at m/z 295.0282 (0.005-Da mass-window width) and 217.0171. Low-energy (full-scan analysis) (b) and High-energy (bbCID mode) (c) spectra and structures of 4-BenzPh Met6 and fragment ion observed. (d) XICs at m/z 248.0717 (0.005-Da mass-window width) and 204.0815. Low-energy (full-scan analysis) (e) and High-energy (bbCID mode) (f) spectra and structures of OC Met1 and fragment ion observed.

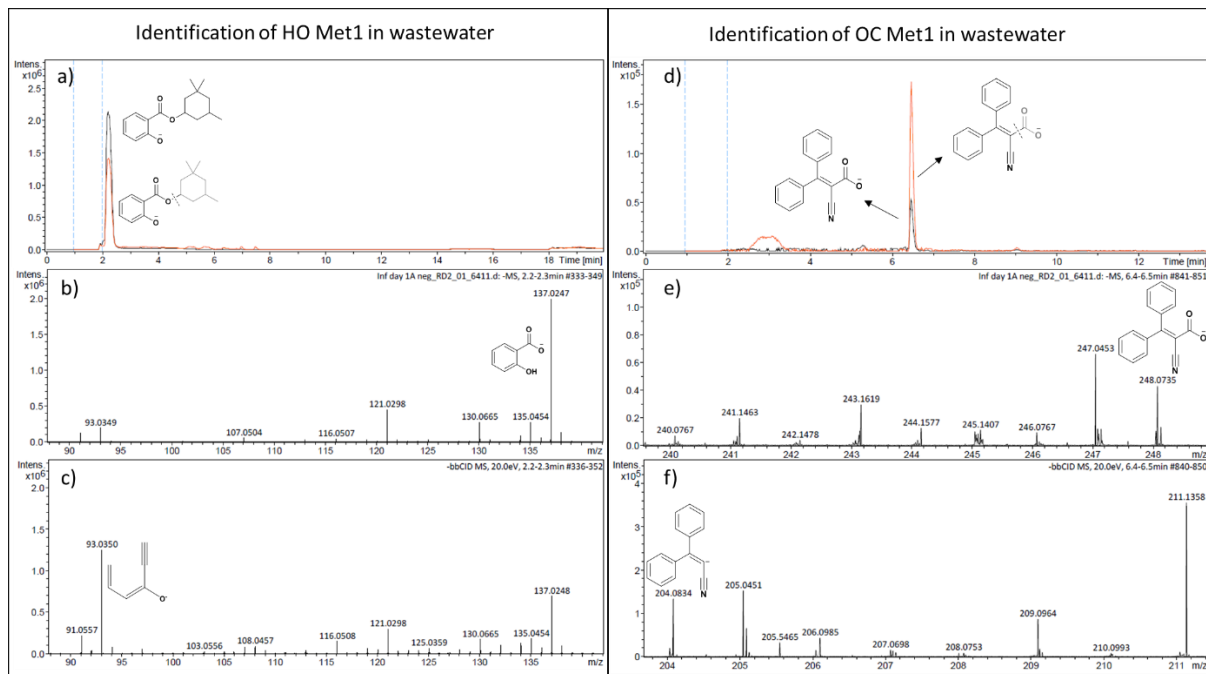


Figure 2 Detection and identification of HO Met1 (a;b;c) and OC Met1 (d;e;f) by UHPLC-QTOF-MS following wastewater fingerprinting assay. (a) XICs at m/z 137.0244 (0.005-Da mass-window width) and 93.0350. Low-energy (full-scan analysis) (b) and High-energy (bbCID mode) (c) spectra and structures of HO Met1 and fragment ion observed. (d) XICs at m/z 248.0717 (0.005-Da mass-window width) and 204.0834. Low-energy (full-scan analysis) (e) and High-energy (bbCID mode) (f) spectra and structures of OC Met1 and fragment ion observed.

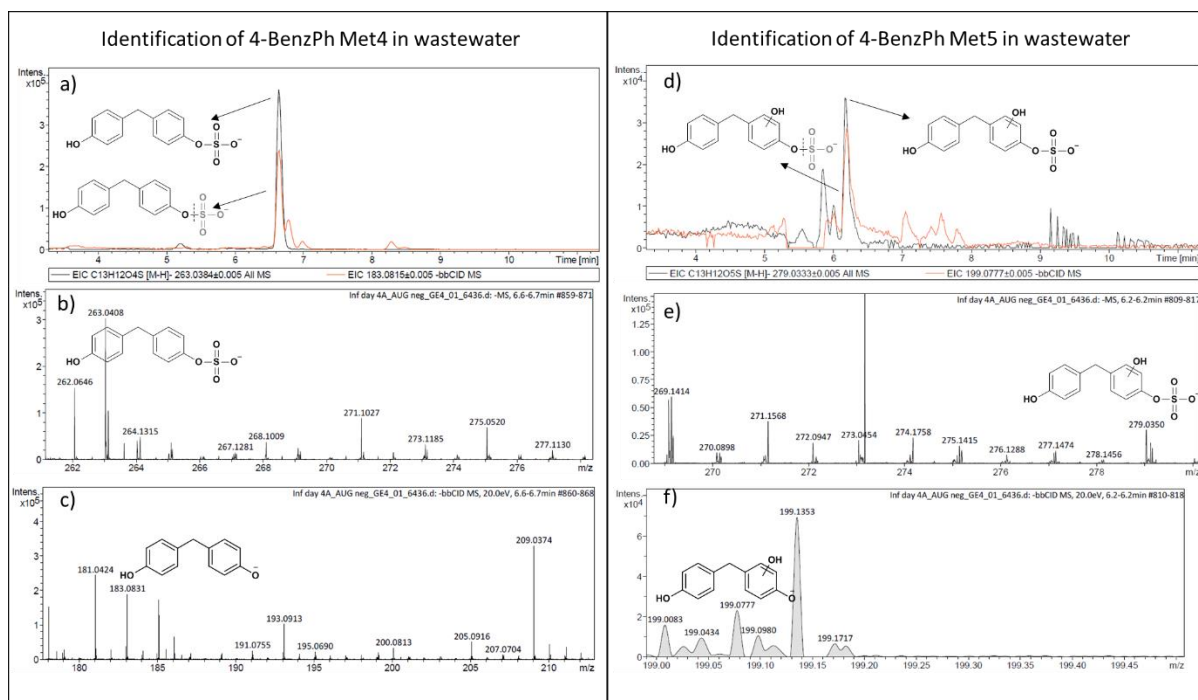


Figure 3 Detection and identification of 4-BenzPh Met4 (a;b;c) and 4-BenzPh Met5 (d;e;f) by UHPLC-QTOF-MS following wastewater fingerprinting assay. (a) XICs at m/z 263.0384 (0.005-Da mass-window width) and 183.0815. Low-energy (full-scan analysis) (b) and High-energy (bbCID mode) (c) spectra and structures of HO Met1 and fragment ion observed. (d) XICs at m/z 279.0333 (0.005-Da mass-window width) and 199.0765. Low-energy (full-scan analysis) (e) and High-energy (bbCID mode) (f) spectra and structures of OC Met1 and fragment ion observed.

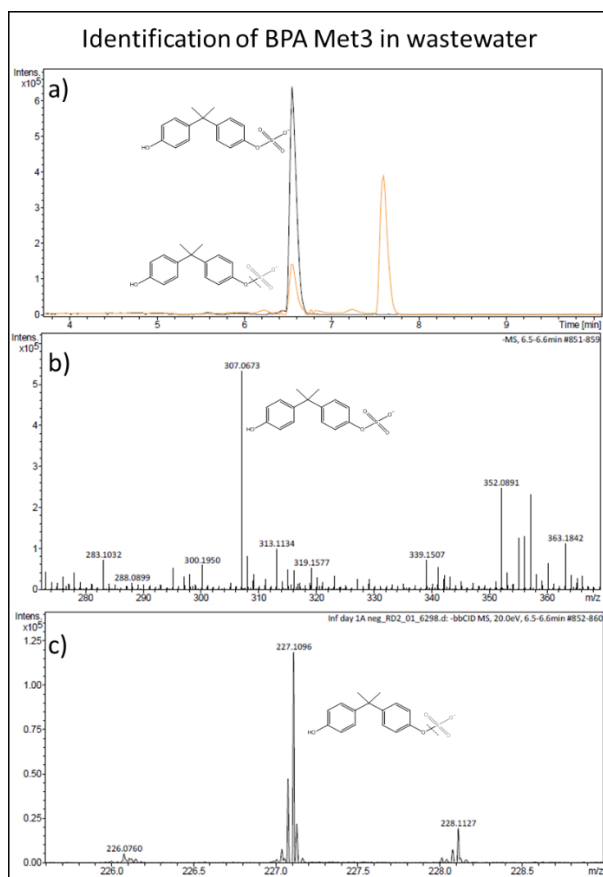


Figure 4 Detection and identification of BPA Met3 by UHPLC-QTOF-MS following wastewater fingerprinting assay. (a) XICs at m/z 307.0646 (0.005-Da mass-window width) and 227.1096. (b) Low-energy (full-scan analysis) and (c) High-energy (bbCID mode) spectra and structures of BPA Met 3 and fragment ion observed.